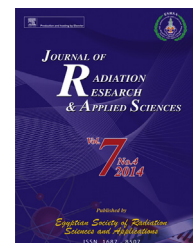


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Effect of recurrent irradiation on the improvement of a variant line of wild tomato (*Solanum pimpinellifolium*)

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ABSTRACT

Solanum pimpinellifolium L. a breed of wild tomato is rich in lycopene. It possess traits which can be transferred to cultivated varieties. Its fruit size is a major hindrance to its domestication. This breed of tomato is very small and thus this work was carried out to improve the size and other desirable traits of the variety. A variant line, SP 300/30.4.2.4, selected from second generation (M2) following irradiation of seeds of *S. pimpinellifolium* L. at 300 Gy was used for the work. 2000 seeds were re-irradiated at 150 Gy and 300 Gy for each treatment and nursed immediately. Plant height at first flowering was highest among the control plants reaching a maximum of 47 cm compared to plants irradiated at 150 Gy and 300 Gy which reached 37 cm and 36 cm respectively. Irradiation therefore led to a reduction in plant height of treated plants. Irradiated materials produced bigger fruits than the controls. The highest mean fruit weight recorded for 300 Gy treated plants was higher than those for 150 Gy and the controls. Variations were observed in the fruit size, shape, colour, plant architecture, number of days to 50% fruiting and flowering. The variations observed could be used selected for and used in subsequent breeding work.

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1. Introduction

Wild tomato (*Solanum pimpinellifolium* L.) belongs to the family Solanaceae. It is a close relative of the commercial variety, *Solanum lycopersicon* L. (Cox, 2000) and may have originated from Western South or Central America (Taylor, 1986). Selection over many generations presumably led to increased fruit size and higher ratio of fruit weight and seed content, characteristics that are typical of the modern day tomato. It is useful for its drought and disease. It has the better ability to

grow under dry conditions than the commercial tomato (*S. lycopersicon* L.), because it naturally grows in places with less water.

The fruits of *S. pimpinellifolium* L. are red, round (Tanksley, 2004) tastes sweet but small, weighing only a few grams, compared to the cultivated varieties which are bigger (Cox, 2000). The fruits contain lycopene, one of the most powerful natural antioxidants. Lycopene is responsible for the deep red colour in tomato fruits (Cox, 2000). In cooked tomatoes, lycopene has been found to help prevent prostate cancer (Clinton et al., 2007) and improve the skin's ability to protect it against

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harmful Ultra Violet (UV) rays. *S. pimpinellifolium* L. contains over 40 times more lycopene than domesticated tomatoes (Cox, 2000).

It is grown by plant breeders and used to improve flavours of commercial varieties of tomatoes. The narrow genetic base of cultivated tomato has resulted in breeders and plant pathologists relying on closely related wild species for allelic variation and thus, the wild relatives of tomato have been used extensively as sources of alleles to improve cultivated tomato germplasm (Osborn, Alexander, & Fobes, 1987).

The use of mutations can result in changes in the growth habit, plant height, oil content, flower colour and leaf shape. Additionally, traits such as leaf shape, texture and colour of a crop may be observed. The floral morphology, fruit size, shape and colour can also be enhanced. In a review, Rick (1976) summarized a number of improvements obtained through breeding to include increase yields by way of larger fruit size and increased fruit number. It also included improvement in fruit quality involving shape, texture, colour and flavour. Plant habit was also modified to facilitate cultural and harvest operations; particularly exploitation of determinate growth habit conditioned by the SP gene (Rick, 1976). There was improvement in handling and storage durability (but sometimes to the detriment of culinary quality). Pest resistance to various species of insects, viral and fungal parasites and nematodes was also improved.

Recurrent irradiation provides an even greater range of genetic variability than would a single irradiation (Khadr & Frey, 1965). Results from various reports present contradicting values of the effect of additional variability induced by recurrent irradiation. Oat (*Avena sativa*) populations developed by recurrent irradiation with thermal neutrons showed expanded variability for quantitative traits over either the original or the pedigree population although the second irradiation cycle did not generate as much variability as the first (Khadr & Frey, 1965). It has been documented that several early examples of chrysanthemum cultivars have been obtained as a result of recurrent irradiation (Broertjes, Koene, & van Veen, 1980; Micke, Donini, & Maluszynski, 1990).

The results of application of radiation include the reduction in yielding capacity of early maturing mutants in barley (Gaul & Mittelstenschied, 1960; Gustafsson, Hagberg, & Lundqvist, 1960). Early maturing mutants especially slightly early maturing ones with yield capacities equivalent to or higher than their original varieties have been induced in several crops (Aastveit, 1965; Kawai, 1963; Porsche, 1963).

Plant height changes in early maturing mutants and significant correlations between the two characters have also been reported in rice (Abrams & Frey, 1964).

The objective of the study was to determine the effect of recurrent irradiation on the improvement of fruit quality (fruit colour, shape and size) and plant architecture of a variant of wild tomato (*S. pimpinellifolium* L.).

2. Materials and method

2.1. Study site

The study site was the experimental farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC). The work was conducted between August 2009 and August, 2010. The farms is located about 20 km north of Accra (05° 40' 60 N and 0° 13' 0 W), with an elevation of 76 m above sea level. The vegetation is Coastal Savannah, and characterized by a bimodal rainfall pattern. The mean annual rainfall is 810 mm distributed over less than 80 days, and temperatures are moderate with maxima rarely exceeding 32 °C while the minimum does not fall below 17 °C.

2.2. Planting materials

Dried seeds of SP 300/30.4.2.4, a variant line selected from second generation (M_2) following irradiation of seeds of *S. pimpinellifolium* L. at 300 Gy from a ^{60}Co gamma source were used. SP 300/30.4.2.4 had an increased fruit size of 2.84 g in relation to the wild parent (Quartey, 2010). The seeds were re-irradiated at 150 Gy and 300 Gy from a ^{60}Co gamma source at 11.88 Gy per hour at position 50 from a radiation source. Irradiation was done at the Radiation Technology Centre (RTC) of the Ghana Atomic Energy Commission, (GAEC) Kwabenya. Non irradiated seeds were used as control (parents).

2.3. Raising of M_2M_1 and M_2M_2 generation

Two thousand (2000) seeds were irradiated for each treatment and nursed immediately after radiation to raise M_2M_1 population. The seedlings were transplanted onto the field after 21 days. The experimental design used was complete randomised design with four replications. Each plot had 210 seedlings with 70 plants of each of the seedlings irradiated at 150

Table 1 – Plant height at first flowering and days to 50% flowering in M_2M_1 and M_2M_2 generation of *S. pimpinellifolium* L. variant line (SP 300/3.4.2.4) following recurrent irradiation.

Trait	Range		Mean \pm SE	
	M_2M_1 population	M_2M_2 population	M_2M_1 population	M_2M_2 population
Plant height at 1st flowering				
0	20.4–47.0	40.3–60.5	27.7 \pm 3.1b	47.4 \pm 5.7a
150	28.3–37.0	38.4–50.7	32.4 \pm 2.7a	43.5 \pm 3.5b
300	26.5–36.0	38.3–47.4	31.1 \pm 3.2a	42.3 \pm 3.3b
Days to 50% flowering				
0	43.0–54.0	45–60	48.7 \pm 4.9a	44.4 \pm 18.9a
150	41.0–50.0	47–55	46.2 \pm 3.2a	51.7 \pm 2.4a
300	40.0–52.0	36–43	43.6 \pm 6.0a	40.0 \pm 2.4a

Table 2 – Fruit weight in the M_2M_1 and M_2M_2 generation of *S. pimpinellifolium* Variant line (SP 300/30.4.2.4) following recurrent irradiation.

Mean fruit weight	Range		Mean \pm SE	
	M_2M_1	M_2M_2	M_2M_1	M_2M_2
0	1.07–1.36	1.35–2.16	1.2 \pm 0.1c	1.7–0.2b
150	0.83–1.70	1.86–2.47	1.6 \pm 0.1a	2.2–0.2a
300	0.90–1.77	1.95–2.66	1.5 \pm 0.1b	2.3–0.1a

Table 3 – Days to 50% fruiting in the M_2M_2 population.

Trait	Range	Mean \pm SE
Days to 50% Fruiting		
0 Gy	52–62	59.2 \pm 5.7a
150 Gy	47–55	51.8 \pm 2.3b
300 Gy	50–53	51.7 \pm 0.9b

Gy and 300 Gy. 100 non-irradiated seeds were used as control. At maturity, plants were harvested and seeds removed to raise the M_2M_2 population. In the M_2M_2 population, 600 seedlings were raised from the nursery and transplanted onto the field. Complete randomised design was used, with 180 seedlings per plot for the seeds irradiated at 150 Gy, 300 Gy and control respectively.

Data were collected on the following; days to first flowering, plant height at 50% flowering, plant architecture, fruit colour, fruit weight, fruit diameter (across and along radial), number of locules per fruit and number of seeds per fruit. Fruit colour was determined using the American Standard Colour chart.

Plant architecture was determined by the branching type of the plant. The erect and less branching plants were said to be determinate or erect while the well branched and crawling plants were assigned indeterminate. Fruits were harvested and weighed on a weighing balance to determine the fruit weights. Fruits with increased weights were selected.

At maturity fruits from each surviving plant exhibiting useful traits such as change in plant architecture, increased fruit weight and fruit colour change were harvested and kept separately.

Data were analysed using One-way ANOVA. SPSS statistical tool was used to analyse the data obtained.

3. Results and discussion

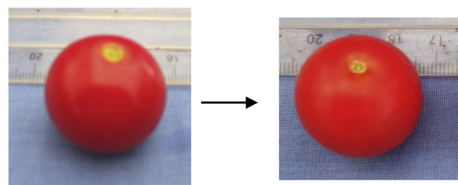
3.1. Plant height at first flowering and days to 50% flowering

Plant height at first flowering among the M_2M_1 population was highest among the control plants reaching a maximum of 47 cm compared to plants irradiated at 150 Gy and 300 Gy which reached 37 cm and 36 cm respectively. Plant height at first flowering for 150 Gy treated plants was similar to that recorded for plants irradiated at 300 Gy (Table 1). However, both were statistically ($p \geq 0.05$) lower than the controls (Table 1). Irradiation therefore led to a reduction in plant height of treated plants. Plants treated at 300 Gy were the first to attain 50% flowering at 40 days while the control was the last after 52 days. Among the 300 Gy plants, it took 40 days for the first plant to flower compared to 41 and 43 days for the 150 Gy and control plants (SP 300/30.4.2.4) respectively (Table 1).

Among the M_2M_2 populations, the control plant (SP 300/30.4.2.4) recorded the highest plant height at 60.5 cm compared to 50.7 cm and 47.4 cm for 150 Gy and 300 Gy treated plants respectively. Statistically ($p \geq 0.05$), treated plants had similar plant heights but different significantly ($p < 0.05$) from the controls. Therefore re-irradiation of the material produced significant reduction in plant height (Table 1). 300 Gy treated plants were the earliest to flower after 36 days of sowing compared to 47 and 45 days for 150 Gy and the controls respectively. Statistically ($p > 0.05$), the controls, 150 Gy and 300 Gy plants had similar days to 50% flowering (Table 1). Irradiation did not produce significant ($p > 0.05$) differences in flowering between the untreated and the treated material.

Table 4 – Characteristics of some variant lines in M_2M_1 generation of *S. pimpinellifolium* Variant (SP 300/30.4.2.4) following recurrent irradiation.

ID No.	Fruit weight (g)	No. of seeds/fruit	No. of locules/fruit	Flesh thickness (mm)	Fruit diameter (cm)	
					Across radial	Along radial
Control	1.36	46	2	1	12	13
M_2M_1 (150 Gy) 144*5	1.70	43	2	1	16	17
M_2M_1 (150 Gy) 90*12	1.67	45	2	1	15	16
M_2M_1 (150 Gy) 90*2	1.63	42	2	1	15	15
M_2M_1 (150 Gy) 93*5	1.51	50	2	1	15	16
M_2M_1 (150 Gy) 52*8	1.08	38	2	1	15	15
M_2M_1 (150 Gy) 55*8	0.83	42	2	1	15	16
M_2M_1 (150 Gy) 2*3	0.91	34	2	1	15	16
M_2M_1 (300 Gy) 1*1	1.77	33	2	1	15	16
M_2M_1 (300 Gy) 32*9	1.41	50	2	1	14	15
M_2M_1 (300 Gy) 2*1	0.90	35	2	1	14	15
M_2M_1 (300 Gy) 13*6	1.58	42	2	1	15	16
M_2M_1 (300 Gy) 32*4	1.50	38	2	1	15	16
M_2M_1 (300 Gy) 103*1	1.26	30	2	1	15	16



(a) SP 300/30.4.2.4 (Variant line)

(b) 300/1*1 (fruit of 300 Gy treated plant)

Fig. 1 – Comparison of fruit size of (a) Variant line (SP 300/30.4.2.4) and (b) biggest fruit weight (300/1*1).

Plant height is controlled by the over dominance type of gene action (Chowdhry, Ambreen, & Khaliq, 2002; Uma & Sharma, 1997). Plant height at 150 Gy and 300 Gy were statistically ($p > 0.05$) similar but different from the controls in M_2M_1 and M_2M_2 generations. Plant height decreased with increasing dose. The difference in the plant height between the treated and controls may be due to the sensitivity of tomato to higher doses (Jamie, 2002). Differences in plant height among the treated and the control may also be attributed to damage to the process of cell division and cell elongation that generally result after mutagenic treatment (Iqbal, 1969).

Days to 50% flowering decreased with radiation but the decrease was not statistically ($p \leq 0.05$) significant (Tables 1 and 4) among treatments. Flowering among plants depends on exogenous (light and temperature) and endogenous (age of plant and hormones). The variants are of the same age, therefore flowering time among treated and parent plants were not statistically significant. This is supported by results of work done by Frydenberg and Sandfaer (1965), who found no differences between the population of barley materials which had been previously irradiated and non-irradiated materials.

3.2. Fruit characteristics in the M_2M_1 and M_2M_2 generation

Mean fruit weights for treated plants and control were statistically ($p \leq 0.05$) different in the M_2M_1 generation. Irradiated

materials produced bigger fruits than the controls (Table 2). The highest mean fruit weight recorded for 300 Gy treated plants was higher than those for 150 Gy and the controls. Therefore the higher dose used for re-irradiation resulted in increased fruit weight (Fig. 1). The highest fruit weight among the controls was 1.36 g compared to 1.70 g (M_2M_1 ; 144*5) and 1.77 g (M_2M_1 ; 1*1) among the 150 Gy and 300 Gy treated plants respectively (Table 2). Fruit weight values for 150 Gy treated plants were higher than those recorded for plants irradiated at 300 Gy (Table 4). However, the highest fruit weight was recorded among 300 Gy irradiated plants. The control plant (SP 300/30.4.2.4) from which the variant lines (treated plants) were obtained had a fruit weight of 2.84 g which was bigger than its progeny and the treated plants. Number of seeds for both sets of treated plants was higher than the controls (Table 4).

M_2M_2 mean fruit weight was similar between 150 Gy and 300 Gy treated plants but differed statistically ($p < 0.05$) from the controls (Table 2). Among the selected variants, fruit weights of most of the 300 Gy treated plants were higher than those of 150 Gy treated plants and the controls. There was no difference in days to 50% flowering for treated plants but these were different from the control plants (Table 2). Selected variants fruited earlier than the untreated material (Table 2). Table 5. Fruit weight and days to 50% fruiting in the M_2M_2 generation of *S. pimpinellifolium* Variant line (SP 300/30.4.2.4) following recurrent irradiation.

Fruiting among the M_2M_2 generation was similar for 150 Gy and 300 Gy but different (statistically) from the controls

Table 5 – Characteristics of some variants in M_2 generation of *S. pimpinellifolium* Variant line (300/30.4.2.4) following recurrent irradiation.

ID No.	Fruit weight (g)	No. of seeds/fruit	No. of locules/fruit	Flesh thickness (mm)	Fruit diameter (mm)	
					Across radial	Along radial
Wild variant (SP 300/30.4.4)	1.67	48	2	1	14	15
M(150 Gy) 52*2	2.47	43	2	1	17	17
M(150 Gy) 52*9	2.45	45	2	1	15	16
M(150 Gy) 52*3	1.96	59	2	1	15	16
M(150 Gy) 52*7	2.41	51	2	1	15	16
M(150 Gy) 55*1	1.86	48	2	1	15	16
M(300 Gy) 1*8a	2.66	55	2	1	15	17
M(300 Gy) 103*14	2.57	40	2	1	14	16
M(300 Gy) 32*3	2.55	45	2	1	15	16
M(300 Gy) 12*5	2.57	95	4	1	20	33
M(300 Gy) 1*8b	1.95	30	2	1	15	16
M(300 Gy) 12*5	3.57	95	4	1	20	33

Note: M (Dose rate), plant number * branch number.

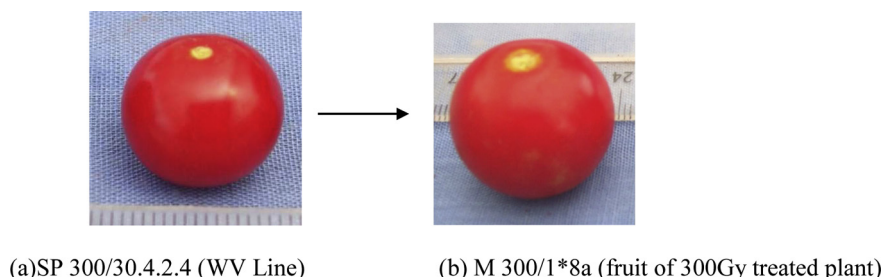


Fig. 2 – Comparison of fruit size of the (a) Wild variant line (SP 300/30.4.2.4) and (b) variant line (M 300/1*8a) in the M_2 generation.



Fig. 3 – Fused variant fruit (M_2M_2 300/12*5).

(Table 4). The earliest to fruit were found among 300 Gy treated plants at 47 days after sowing. Plants irradiated at 300 Gy that fruited earlier may be due to the early flowering of the higher doses resulting in early fruiting. Therefore, plants irradiated at 300 Gy which flowered earlier, fruited earlier compared to the 150 Gy and the control. Also it may be due to the expanded genetic variability obtained from recurrent irradiation from which further variability was obtained (Khadr & Frey, 1965).

Fruit weight is a quantitatively inherited character and is controlled by many genetic loci, some with a large effect and others with a small effect (Ben-Chaim et al., 2006; Doganlar, Frary, Daunay, Lester, & Tanksley, 2002; Grandillo, Ku, & Tanksley, 1999). High fruit weights observed may be due to

effect of irradiation on the allele *fw 2.2* which influences fruit weight (Frary et al., 2000). This allele acts as a regulator of cell division in larger size fruits of tomato. The differences in fruit size observed may be due to the regulators of cell division and cell size acting after anthesis (Paran & van der Knaap, 2007).

The variant M_2M_2 52*2 gave the highest fruit weight of 2.47 g among the 150 Gy plants while the variant M_2M_2 1*8a gave the highest fruit weight of 2.66 g among the 300 Gy plants. Variant M_2M_2 (150 Gy) 52*3 had the highest seed number of 59 (Table 5). Locules number and flesh thickness were similar for the controls and treated plants. The fruit with the highest fruit weight had the highest diameter of 17 mm across and along the radial of the fruit (Table 6).

Plants selected from the M_2M_1 generation with erect architecture changed to branching type. M_2M_2 plants derived from yellow fruit-bearing M_2M_1 control produced a mixture of chimeric and red fruits.

The variant M_2M_2 12*5 among the 300 Gy plants produced a fused fruit of weight 3.57 g. It had 4 locules and the diameter across the radial was 20 mm and along it was 33 mm and contained 95 seeds.

Flesh thickness and number of locules were the same for all the plants irrespective of dose of re-irradiation applied. The fruit diameter of the treated plants was higher than the controls. For control plants, fruit diameter across and along the radial section was 12 mm and 13 mm respectively and for treated plants, 15 mm and 17 mm (Table 3). Irradiation did not have any effect on the number

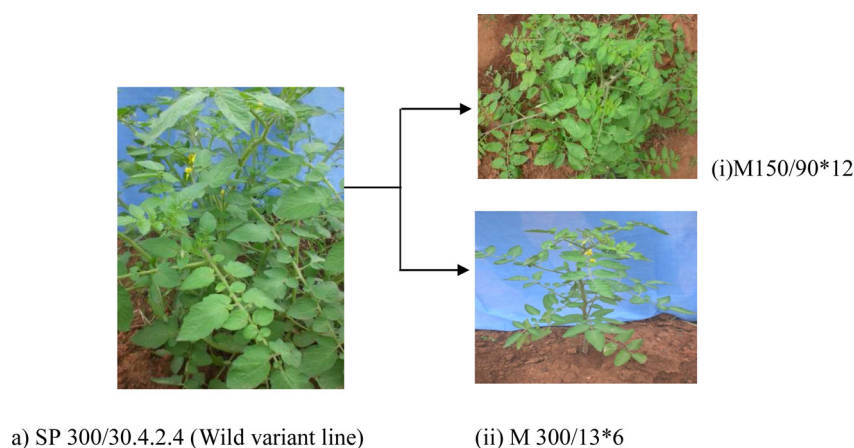


Fig. 4 – Variation in plant architecture: (a) Wild variant plant exhibiting indeterminate plant structure. (i) 150 Gy variant plant exhibiting indeterminate plant structure (ii) 300 Gy variant plant exhibiting determinate plant structure.

of seeds, locules and flesh thickness among the various treatments (Table 3).

3.3. Variation in fruit colour and plant architecture in the M_2M_1 generation of *S. pimpinellifolium* variant line (SP 300/30.4.2.4) following recurrent irradiation

Among 238 variant lines obtained from re-irradiating material at 150 Gy, thirty-three (33) exhibited yellow fruit colouration and 16 variants were found to produce fruits for traits of patched fruits and one all-yellow fruit bearing plant among the 300 Gy treated plants.

Variations were observed for plant architecture among the variant lines. Three (3) variant lines of 150 Gy and two (2) variant lines among 300 Gy treated plants were found to exhibit determinate (erect) plant structure (Fig. 4). The other variants for both treatments exhibited an indeterminate plant architecture (branching or wild type) which is characteristic of the controls.

Variations in the fruit colour observed may be due to mutations in the enzymes of the carotenoid biosynthetic pathway. The red colour of wild tomato results from the accumulation of carotenoid pigments. Green unripe fruits of tomato contain chlorophyll and carotenoid pigments such as lutein, β -carotene and violaxanthin. When ripened, the chloroplasts are converted into chromoplasts which results in the red colour of mature fruits. Yellow coloured tomato is controlled by the locus yellow flesh (R) (Frery & Grierson, 1993). Yellow coloured fruits found among some variant lines may be due to low amounts of yellow carotenoid such as lutein and flavonoids in the skin (Paran & van der Knaap, 2007). Fruit colour changes obtained from both generations includes an all-yellow peduncle fruit and patches of yellow and red (in the web version). The chimeric nature of the fruits may be due to the effects of gamma irradiation on the genes that code for specific colour traits. The fruit colour changes observed could also be due to mutations in genes from other pathways which had influenced the intensity of the fruit colour, resulting in patches on fruits (Paran & van der Knaap, 2007).

Extensive vegetative and axillary growth is observed in wild tomato compared to the cultivated varieties where growth is not extensive. Genetic inheritance of the extensive growth of the wild plant is not well known. The erect nature of some of the variant lines may be due to the locus SP that affects tomato plant stature. Plants possessing the locus SP have their sympodial units terminated at an earlier stage and thus resulting in less branching and producing an erect plant or compact plants (Pneuli et al., 1998).

4. Conclusion

Recurrent irradiation, radiation after subsequent generations in an already irradiated material is meant to expand an already created genetic variability. The application of this process to SP 300/30.4.2.4, an M_2 variant line of *S. pimpinellifolium* resulted in the generation of useful traits. Changes in plant architecture and number of days to 50% fruiting were recorded in the M_2M_1 generation. Reduced plant height and

earliness that would lead ultimately to agronomically acceptable plant architecture and early variants as well as the use of such a variant in cross breeding programmes are good prospects.

Fruit size increase as well as fruit colour changes were obtained. These are variants that could be developed into mutant varieties and/or used in cross breeding programmes.

A variant line was obtained with fused fruit and had four locules resulting in higher fruit weight (size). This fruit can be used for crossing to enhance the size of hybrids in subsequent generations.

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